

The University of Chicago

PHOTOPERIODIC
INDUCTION AS INFLUENCED BY
ENVIRONMENTAL FACTORS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1939

By
ELBERT MONROE LONG

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PHOTOPERIODIC INDUCTION AS INFLUENCED BY ENVIRONMENTAL FACTORS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 504

ELBERT M. LONG

(WITH TWO FIGURES)

Introduction

Xanthium pennsylvanicum may be photoperiodically induced by exposure to a single dark period of 15 hours at 70° F., even though subsequently grown on photoperiods not directly conducive to floral initiation. Since several lines of evidence (5) indicate that photoperiodic induction is determined by length of the dark period, it is perhaps more appropriate, with this plant, to apply the term "critical dark period" rather than "critical day" or "critical photoperiod," as has been applied to some other plants (4). Length of the critical dark period for *Xanthium* is $8\frac{1}{2}$ to 11 hours, depending upon environmental factors, of which temperature seems to be the most effective.

It has been demonstrated (1, 2, 3, 5, 6, 7, 8, 9, 10, 11) that the leaves of several species of plants, including *Xanthium*, perceive the photoperiodic stimulus. Whether the leaves are the locus of photoperiodic induction is not known, but indications from the present work are that this is the case. Since the leaves perceive the photoperiodic stimulus, and since usually the stimulation occurs with exposure to 9 hours of darkness and not 8 hours, an attempt was made to determine what environmental factors influence the length of dark period necessary for induction. This seemed particularly pertinent since *Xanthium* during the winter months may respond variously, sometimes a particular treatment resulting in flowering and at other times not. The environmental factors which obviously change considerably from summer to winter in the greenhouses at Chicago are light intensity, temperature, and humidity; therefore the effect of variation in these factors upon length of the critical dark period was investigated.

¹ This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

General methods

The cocklebur, *Xanthium pennsylvanicum*, was used for most of these experiments. Plants were grown from seeds collected in the vicinity of Chicago in November. Until desired for use, the fruits were stored in a bin out of doors, exposed to variations of weather. The seeds were then removed from the fruits, scratched slightly to rupture the coats, and planted within a few days in a light garden soil in ordinary flats. The seedlings were transplanted singly to pots when they had attained heights of 4-6 inches. Before the seeds germinated, the flats were placed on a well lighted bench in the greenhouse where supplementary illumination was provided by Mazda light of approximately 100 foot candles at the soil surface from sun-down until 2:00 A.M. (For convenience, these conditions are referred to here as the long photoperiod.) This photoperiod varied considerably during the course of the work, but was never less than 18 hours in duration. *Xanthium* plants kept continuously under these conditions remained strictly vegetative, some for nearly a year, showing no sign of flower initiation when dissected. Biloxi soybeans were also used as experimental material. The seeds were planted in fertile soil at the rate of four per 5-inch clay pot. The pots were placed on long photoperiod and watered regularly until used for experiment.

Excessively high temperatures and low humidities were minimized by careful ventilation of the greenhouse and frequent sprinkling of the walls and benches. The plants were watered frequently with tap water, and under optimum conditions for growth, as often as twice weekly with a double strength of Shive's (12) R_2S_5 nutrient solution. Unless otherwise stated, the plants grew vigorously throughout the experimental period. In general, after a given treatment the plants were returned to long photoperiod and allowed to grow until harvested.

Except when clearly evident macroscopic flowers developed, flowering response of cockleburs and soybeans was determined by careful dissection under a binocular microscope with a magnification of 20.7. Usually only the terminal buds of cocklebur were dissected, as in that species the terminal bud is almost invariably the first to initiate floral primordia, and therefore is considered the most depend-

able one in determining flowering response. With soybean it was necessary to dissect all the buds, for the terminal meristem produces floral parts only after a number of days of treatment (1), while in the vicinity of the fifth node from the terminal meristem they are differentiated 3-5 days after first being subjected to short photoperiod.

Investigation

RESIDUAL EFFECT IN LEAF RESULTING FROM PHOTOPERIODIC TREATMENT

Various experiments have shown (5) that a single photoperiodically induced intact leaf of *Xanthium* may effect floral initiation in a



FIG. 1.—A, induced leaf grafted to vegetative plant, 4 weeks after grafting. As a result of the graft the plant has initiated floral primordia. B, enlarged view of grafted leaf.

non-induced plant when the plant is grafted to the one bearing the induced leaf. It might appear that such a leaf, after having been induced, could be removed from the plant on which it was borne and

be transferred to a vegetative non-induced plant with the result that the vegetative plant would flower because of a residual stimulus or substance transmitted to it from the leaf grafted upon it. To test this possibility, six *Xanthium* plants were subjected to two consecutive 15-hour dark periods separated by a photoperiod during which induction was completed. During the photoperiod following the second long dark period, two leaves from each of the treated plants were removed by severing the petiole obliquely across the axis about 0.5 inch from the stem. Two leaves of each of six vegetative plants which had been maintained on long photoperiod were similarly removed and the leaves from the induced plants grafted to the vegetative ones, all being subsequently maintained on long photoperiod.

TABLE 1
EFFECT OF GRAFTING LEAVES OF INDUCED PLANTS TO VEGETATIVE PLANTS

TREATMENT	NO. OF PLANTS	FLOWERING CONDITION AFTER FOUR WEEKS
Two leaves from photoperiodically induced plants grafted to each vegetative plant. . . .	6	3 with flower primordia 3 vegetative
Two leaves from vegetative plants grafted to each vegetative plant (controls).	6	6 vegetative

The contact was accomplished by placing one end of a short piece of glass tubing over the cut stump of the petiole of the vegetative plant and inserting the cut end of the leaf into the other end of the tube in such a way that the two cut surfaces were in intimate contact (fig. 1). The glass tube served to support the leaf and hold the cut surfaces in close contact. The plant was then placed in a nearly saturated atmosphere in a glass chamber and exposed to long photoperiod. To serve as controls for these six experimental plants, leaves from six vegetative plants were removed and grafted to six other vegetative plants in a similar manner. These control plants were maintained by the side of the experimental plants. The results are shown in table 1.

Although only three of the treated plants initiated flowers, these were the ones on which the grafted leaves remained alive for practically the entire period of the experiment (fig. 1). Most of the grafted leaves died within 10 days after grafting.

Leaves may be photoperiodically induced and subsequently may supply substances leading to floral initiation under conditions of long photoperiod or in whole plants no part of which has undergone any other type of inductive treatment.

RESIDUAL EFFECT OF TREATMENT WITH SHORT PHOTOPERIOD
INSUFFICIENT TO BRING ABOUT PHOTOPERIODIC
INDUCTION

In the subsequent discussion relating to subjection of plants to dark periods of varying length, unless otherwise stated a long dark period is one of 15 hours accompanied by a 9-hour photoperiod, and a short dark period one of 8 hours accompanied by a 15-hour photoperiod. Although *Xanthium* may be induced to flower when subjected to a single dark period at 70° F., as many as eight long dark periods may be required if the temperature during the dark period is 40° F. Biloxi soybean may initiate flower primordia as the result of exposure to three long dark periods alternating with short photoperiods at 70°. When more than a single long dark period is required for induction, it is possible that there may be a residual effect of each of the dark periods, which being cumulative finally results in the induced condition. In order to test such a possibility the following experiments were carried out.

On April 1, 192 vegetative *Xanthium* plants were selected for uniformity and exposed to two long dark periods at 40° F., the two periods being separated by a short photoperiod in the greenhouse at 70°. They were then returned to long photoperiod at 70°. Sixty more were selected to serve as initial controls and divided into ten lots of six plants each. One lot was exposed to one long dark period at 40° F., one lot to two long dark periods at 40°, and so on, the last lot receiving a total of ten long dark periods at 40°, each dark period being separated by a short photoperiod at 70° as previously indicated. After final treatment each lot of these initial controls was returned to the greenhouse to continue on long photoperiod until the entire experiment was harvested. Fifty other plants, selected at the same time as final controls, were divided into ten lots of five plants each and treated exactly as were the initial controls except that their

long dark period ran concurrently with that of the last group of the experimental plants.

The original 192 plants, all of which had received two long dark periods at 40° F. when returned to the long photoperiod bench, were divided into four groups of forty-eight plants each. One of these groups remained on long photoperiod for 2 days before further treat-

TABLE 2
FLOWERING RESPONSE OF XANTHIUM PLANTS TREATED WITH
VARIOUS NUMBERS OF 15-HOUR DARK PERIODS AT 40° F.*

TREATMENT RECEIVED PREVIOUS TO EXPOSURE TO 15-HOUR DARK PERIODS	DATES OF LONG DARK PERIOD TREATMENTS	No. of 15-HOUR DARK PERIODS (AT 40°) RECEIVED SUBSEQUENT TO TREATMENT									
		1	2	3	4	5	6	7	8	9	10
No previous treatment (initial controls).....	April 4-13	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl	6 veg	6 veg	5 veg 1 fl	2 veg
Two long dark periods at 40°, then 2 short dark periods in greenhouse...	April 1 & 2; April 5-14	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg
Two long dark periods at 40°, then 4 short dark periods in greenhouse...	April 1 & 2; April 7-16	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg		2 veg 4 fl		1 veg 4 fl
Two long dark periods at 40°, then 8 short dark periods in greenhouse...	April 1 & 2; April 11-20	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl		2 veg 4 fl		 6 fl
Two long dark periods at 40°, then 16 short dark periods in greenhouse...	April 1 & 2; April 14-23	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl		5 veg 1 fl		6 veg
No previous treatment, but maintained on short dark periods until April 14 (final controls).....	April 14-23	5 veg	5 veg	5 veg	5 veg	5 veg	4 veg 1 fl	2 veg 3 fl	3 veg 2 fl	3 veg 2 fl	2 veg 3 fl

* All photoperiods and all dark periods (other than those at 40°) occur in the greenhouse.

ment, a second group remained for 4 days, a third for 8 days, and the fourth for 16 days. Each group was then subdivided into eight lots of six plants each, one lot being exposed to one long dark period at 40° F., one to two long dark periods, one to three, one to four, one to five, one to six, one to eight, and one to ten (table 2).

Although growing conditions in March, just previous to beginning the experiment, had been reasonably favorable for rapid growth, the weather from April 1 to April 20 was very cloudy. It is possible that the variable results are owing to variation in light intensity during the experimental period.

While no definite conclusions could be drawn, indications were that there was not sufficient residual effect of a long dark period at 40° in *Xanthium* to persist through two or more long photoperiods at 70°.

Another experiment was designed to show whether there is any residual effect at all, and if so, over how many long photoperiods it persists. For this experiment, beginning April 26, thirty-six pots of *Xanthium* plants were given an initial treatment of four consecutive

TABLE 3

RESPONSE OF XANTHIUM PLANTS TO INITIAL TREATMENT OF FOUR LONG DARK PERIODS AT 40° FOLLOWED BY EIGHT ADDITIONAL LONG DARK PERIODS EACH INTERSPERSED WITH VARYING NUMBERS OF SHORT DARK PERIODS AT 70° F.

NO. OF LONG DARK PERIODS OF INITIAL TREATMENT	NO. OF SHORT DARK PERIODS INTERVENING BETWEEN ADDITIONAL LONG DARK PERIODS	NO. OF PLANTS WITH FLOWER PRIMORDIA	NO. VEGETATIVE
Four	1	5	1
	2	0	6
	3	0	6
	4	0	6
	5	0	6
Four (controls)	(on continuous long photoperiod after initial treatment)	1	5

15-hour dark periods at 40°. Thereafter, six pots were returned to continuous long photoperiod as controls. Another group of six pots received, after the initial treatment, alternate short dark periods at 70° and long dark periods at 40° until they had had a total of twelve long dark periods. A third group of six got two short dark periods, a long dark period, two short dark periods, a long one, etc., until they had had a total of twelve long dark periods. The fourth, fifth, and sixth groups of six were treated in the same way except that the period of short dark period treatment was progressively one dark period longer in each. Results of this experiment (table 3) indicate that there is some cumulative effect of the long dark periods, but that it diminishes rapidly under the influence of short dark periods.

Those plants which received four long dark periods at 40° , followed by alternating short dark periods at 70° and long dark periods at 40° , produced flowers. On the other hand, none of those plants whose long dark period treatment was interrupted periodically by exposures to two or more short dark periods showed any indication of flowering. Indications are that, in *Xanthium*, a treatment with four long dark periods at 40° produces an effect in the plant, such an effect being carried over for more than 24 hours.

It might be assumed that one dark period at 40° would cause flowering if the dark period were of sufficient length. That this is very unlikely is shown by the following experiment. Forty pots of *Xanthium* were placed in the darkroom at 40° on May 9, and at more or less regular intervals four pots were removed and placed on long photoperiod in the greenhouse. None of the plants flowered even though some of them received 112 hours of continuous darkness. All plants seemed in excellent condition when removed from the darkroom. Apparently, under these conditions of low dark period temperature, photoperiodic induction does not take place unless the long dark periods are accompanied by short photoperiods.

EXPERIMENTS WITH BILOXI SOYBEANS

Experiments similar to those just reported were performed with soybeans. Since for induction, soybeans ordinarily require at least three long dark periods at greenhouse temperatures, it was not necessary to use the refrigerated room for dark period treatments.

On March 22, ninety-six pots, each containing four vegetative Biloxi soybean plants, were selected for uniformity from those growing on the long photoperiod bench. Excepting twenty-four pots reserved for controls, half the plants were given an initial treatment of one 15-hour dark period and half an initial treatment of two 15-hour dark periods. After the initial treatment the plants were removed to the long photoperiod bench, one-third of each group remaining there for 5 days, one-third for 10, and one-third for 20 days. After receiving the specified number of long photoperiods, one-fourth of each group received one additional long dark period; one-fourth, two long dark periods; one-fourth, three long dark periods; and one-fourth, five long dark periods. The twenty-four pots of controls were divid-

ed into two groups to serve as initial and final controls. Beginning March 22, two pots of initial controls were given one long dark period; two pots two consecutive long dark periods; two pots, three; two,

TABLE 4

FLOWERING RESPONSE OF BILOXI SOYBEANS EXPOSED TO EITHER ONE OR TWO PERIODS OF LONG DARK PERIOD TREATMENT AT DIFFERENT STAGES OF GROWTH*

No. OF LONG DARK PERIODS OF EXPOSURE IN INITIAL TREATMENT	No. OF SHORT DARK PERIODS INTERVENING BETWEEN INITIAL AND FINAL TREATMENTS	DATE OF BEGINNING OF FINAL TREATMENT	No. OF LONG DARK PERIODS OF FINAL TREATMENT					
			1	2	3	4	5	7
Initial controls	None	March 22	8 veg 0 fl	7 veg 0 fl	5 veg 2 fl	2 veg 5 fl	7 fl	7 fl
One long dark period on March 22	5	March 28	7 veg	8 veg	8 veg		6 fl	
	10	April 2	8 veg	8 veg	6 veg 2 fl		7 fl 7 fl	
	20	April 12	7 veg	8 veg	7 veg		8 fl	
Two long dark periods on March 22 and 23	5	March 29	7 veg	7 veg	6 veg 1 fl		2 veg 5 fl	
	10	April 3	8 veg	7 veg	8 veg		7 fl	
	20	April 13	6 veg 1 fl	7 veg 1 fl	4 veg 4 fl		1 veg 6 fl	
Final controls	None	April 13	8 veg	7 veg 1 fl	7 veg 1 fl	1 veg 7 fl	8 fl	8 fl

* Plants grown on long photoperiod until March 22, when all were vegetative. After the various treatments all buds were carefully dissected and presence or absence of flower primordia noted. If a plant possessed one or more flower primordia it is reported as flowering (fl); other plants reported as vegetative (veg).

four; two, five; and two, seven. The final controls remained on the long photoperiod bench continuously until April 13, when they began a course of treatment similar to that of the initial controls.

After the treatments with long dark periods, all plants were returned to the long photoperiod bench where they remained for 2 weeks, after which they were carefully dissected to determine whether flowers or flower primordia were present (table 4).

As the plants grew older they required fewer long dark periods for induction. Thus the initial controls, treated March 22, showed some flowering 3 days after treatment and all flowered after 5 days. The final controls, treated April 13, showed some flowering after treatment for 2 days and all but one flowered after 4 days. This indicates that as the plants grow older they become more sensitive to photoperiodic treatment. The results show that the effect of an initial

TABLE 5

RESPONSE OF BILOXI SOYBEANS TO INITIAL TREATMENT OF TWO LONG DARK PERIODS FOLLOWED BY EIGHT LONG DARK PERIODS EACH INTERSPERSED WITH VARYING NUMBERS OF SHORT DARK PERIODS

NO. OF LONG DARK PERIODS OF INITIAL TREATMENT	NO. OF SHORT DARK PERIODS INTERVENING BETWEEN ADDITIONAL LONG DARK PERIODS	NO. OF PLANTS WITH FLOWER PRIMORDIA	NO. VEGETATIVE
Two	1	0	12
	2	3*	13
	3	0	14
	4	0	10
	5	0	14
Controls	(on continuous long photoperiod after initial treatment)	0	6
Two		1	5
Three		5	2
Four		5	1
Five			

* It is possible that these plants were shaded sufficiently by others so that their supplementary illumination on the long dry bench was too low to prevent flowering.

treatment of one or two long dark periods does not persist over as many as five long photoperiods.

Another experiment was carried out to determine the possible residual effect over fewer long photoperiods. For this, twenty-eight pots were selected. Of the eight pots of controls, two received two consecutive long dark periods; two, three long dark periods; two, four; and two, five before being returned to the long photoperiod bench. The other twenty pots all received an initial treatment of two long dark periods, after which they were divided into five groups of four pots each. The first group then received a short dark period, a long dark period, a short one, and so on, until the plants had been

subjected to a total of ten long dark periods, when they were returned to the long photoperiod bench until dissected. The remaining four groups were treated in essentially the same manner except that the period of short dark periods between each of the single long dark periods of the final treatment was progressively one dark period longer for each group. The results of this experiment (table 5) unmistakably indicate that the effect of two long dark periods does not persist for more than 24 hours.

RESIDUAL EFFECT OF TREATMENT WITH SHORT PHOTOPERIOD
SUFFICIENT TO BRING ABOUT PHOTOPERIODIC INDUCTION

Since these two experiments indicated that there was no appreciable residual effect of long dark period treatments too brief to bring about floral initiation, the question arose as to whether there was an appreciable residual effect provided the initial treatment was sufficient to cause floral initiation. In consequence, a third experiment was carried out.

On March 19, 150 pots each containing four vigorously growing, 1-month old, vegetative Biloxi soybean plants were selected for uniformity from 500 pots on the long photoperiod bench. The various treatments are given in table 6. Those plants treated with long dark periods during the period March 19-24 were placed under the diurnal photoperiod in the greenhouse at that time, without supplementary illumination. Subsequent to March 24 all plants, when exposed to long dark periods, were given dark periods of 15 hours at greenhouse temperatures.

The plants were harvested May 22, at which time eight uniform plants of the sixteen on each experimental treatment were carefully dissected and the presence of flowers at each node on the main axis noted according to the method outlined by BORTHWICK and PARKER (1). As may be seen in table 6, those plants which received the greatest number of long dark periods had the greatest number of flowers.

Treatment with long dark period from the period March 19-30 was not so effective in bringing about floral initiation as were the later periods. This may be related to the fact that the plants were relatively young during the earlier period, or it may be that the long dark periods were not so effective because they were only the natural

night lengths of that time and thus were slightly shorter than 15 hours. It may also be noted that those plants which received two

TABLE 6

EFFECT OF VARIOUS SUCCESSIVE TREATMENTS WITH LONG DARK PERIOD UPON FLOWER BUD FORMATION IN BILOXI SOYBEANS

TREATMENT (PLANTS HARVESTED MAY 22)	NO. OF LONG DARK PERIODS	DATE	NO. OF PLANTS WITH FLOWER PRIMORDIA	TOTAL BUDS ON MAIN AXIS OF EIGHT PLANTS CON- TAINING FLOWER PRIMORDIA
No long dark period until March 19, then:	1	March 19	0	0
	3	March 19-21	0	0
	5	March 19-23	7	11
	6	March 19-24	7	14
	7	March 19-25	8	22
	9	March 19-27	8	26
Six long dark periods* March 19-25, then ten long photoperiods, March 25-April 4, then:	1	April 4	7	13
	3	April 4-6	7	15
	5	April 4-8	8	28
	7	April 4-10	8	39
	9	April 4-12	8	59
No long dark period until April 1, then:	1	April 1	0	0
	3	April 1-3	0	0
	5	April 1-5	5	14
	7	April 1-7	8	31
	9	April 1-9	8	44
Six long dark periods* March 19-25, then twenty long photoperiods, March 25-April 14, then:	1	April 14	7	14
	3	April 14-16	6	15
	5	April 14-18	8	38
	7	April 14-20	8	42
	9	April 14-22	8	60
No long dark period until April 10, then:	1	April 10	0	0
	3	April 10-12	3	6
	5	April 10-14	8	25
	7	April 10-16	8	35
	9	April 10-18	8	41

* These long dark periods were equal to the duration of the natural night during this period and of approximately 13 hours; all other long dark periods were 15 hours.

treatments of long dark periods developed approximately as many flowers as the combined numbers produced on two separate sets of plants, one set receiving a number of long dark periods comparable

with the first treatment of the former plants and the second receiving a treatment comparable with the second treatment.

This relationship is shown more clearly in figure 2. Here three sets of plants are shown diagrammatically. Figure 2*A* shows each

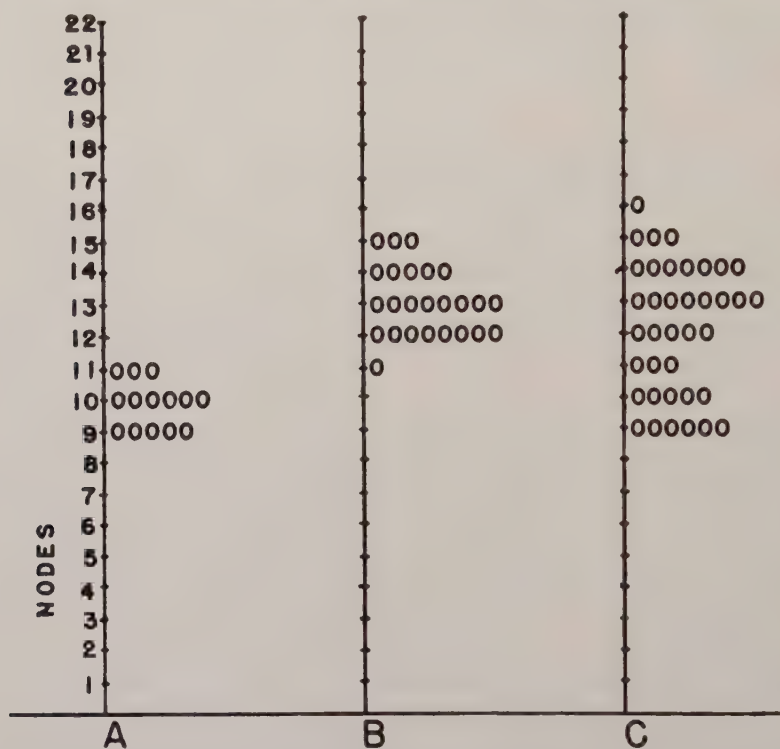


FIG. 2.—Diagrammatic representation of effect on Biloxi soybeans of various long dark period treatments.

flower of the eight plants which received six long dark periods (March 19-26). Each flower is shown in its relative position on the plant. Figure 2*B* illustrates the flowering condition of the eight plants which received five long dark periods (April 10-15). Figure 2*C* illustrates those plants which received both long dark period treatments; that is, six long dark periods (March 19-26) and five long dark periods (April 10-15). It may be seen that *C* represents almost precisely a composite picture of *A* and *B*.

These results all indicate that in soybean an induction period of several long dark periods acts more or less independently of previous treatments if the various treatments are separated by one or more short dark periods.

This is somewhat in contrast to *Xanthium* plants which are exposed to long dark periods at 40° F. In *Xanthium* the residual effect lasted 24 hours or more. In soybean also, even though a plant receives an induction period of sufficient length to bring about floral initiation, in order that more flowers be produced by a subsequent photoperiodic treatment the plant must undergo a second induction period. This is again in contrast to *Xanthium* which, once it is photoperiodically induced, seems to remain more or less permanently so and will continue to produce additional primordia over a long period of time.

EFFECT OF TEMPERATURE ON CRITICAL DARK PERIOD LENGTH OF XANTHIUM PLANTS OF TWO DIFFERENT AGES

Since a low dark period temperature produces a profound effect upon the length of time required for induction of *Xanthium* (5), it was decided to ascertain just what effect temperature exercised upon the critical photoperiod. Results have repeatedly been obtained in this work, and in that of HAMNER and BONNER (5), which indicate that the critical dark period is between 8 and 9 hours with summer growing conditions and dark period temperatures between 70° and 80° F. In the following experiment a determination was made of the critical dark period of plants exposed to photoperiods in the greenhouse and dark periods at 40° F.

Two hundred and forty vegetative plants were selected from among those growing on the long photoperiod bench. One-half were approximately 3 weeks old and about 6 inches in height, possessing three to five fully expanded leaves. The other half were a month older, about 1 foot in height, possessing eight to eleven fully expanded leaves. Each of these two groups was divided into twenty-four lots containing five plants each. Each lot of five young plants was combined with a corresponding lot of older plants, making twenty-five lots each of ten plants for each experimental treatment. Sixteen of these lots then received a treatment of fifteen consecutive dark

periods at 40° F. The first lot was subjected to dark periods of 8 hours, and the others to dark periods progressively longer by 20-minute increments for each lot, the sixteenth lot receiving dark periods of 13 hours. The remaining eight lots received fifteen dark periods

TABLE 7

RESPONSE OF LARGE AND SMALL XANTHIUM PLANTS SUBJECTED TO VARIOUS DARK PERIOD LENGTHS AT TEMPERATURES OF 40° AND 70°

TEMPERATURE DURING DARK PERIODS (°F.)	DURATION OF DARK PERIODS (HOURS)	LARGE PLANTS		SMALL PLANTS	
		NO. WITH IN- FLORESCENCES OR FLOWER PRIMORDIA	NO. VEGE- TATIVE	NO. WITH IN- FLORESCENCES OR FLOWER PRIMORDIA	NO. VEGE- TATIVE
40°	8.....	0	5	0	5
	8 $\frac{1}{2}$	1	4	0	5
	8 $\frac{2}{3}$	0	5	0	5
	9.....	0	5	0	5
	9 $\frac{1}{3}$	0	5	0	5
	9 $\frac{2}{3}$	1	4	0	5
	10.....	0	5	0	5
	10 $\frac{1}{3}$	1	4	0	5
	10 $\frac{2}{3}$	1	4	0	5
	11.....	2	3	1	4
	11 $\frac{1}{3}$	5	0	2	3
	11 $\frac{2}{3}$	5	0	5	0
	12.....	5	0	4	1
	12 $\frac{1}{3}$	5	0	5	0
	12 $\frac{2}{3}$	4	1	5	0
	13.....	5	0	5	0
70°	8.....	0	5	0	5
	8 $\frac{1}{2}$	3	2	0	5
	8 $\frac{2}{3}$	5	0	5	0
	9.....	5	0	5	0
	9 $\frac{1}{3}$	5	0	5	0
	9 $\frac{2}{3}$	5	0	5	0
	10.....	5	0	5	0

at approximately 70° F. The first of these lots was also subjected to 8-hour dark periods, and the others to dark periods progressively longer by 20-minute increments for each lot, the eighth lot being exposed to dark periods of 10 hours and 20 minutes.

The results of this experiment (table 7) indicate that the critical dark period at 40° F. was about 11 hours for the large plants and at least 20 minutes longer for the small plants. Since the critical dark

period of the controls (at approximately 70° F.) proved to be about 8 hours and 20 minutes for the large and 8 hours and 40 minutes for the small plants, the lower temperature increased the critical dark period about 2 hours and 40 minutes.

These results indicate, as has other work (1), that as plants grow older they become more sensitive to photoperiodic treatment. Thus at both temperatures the old plants had a shorter critical dark period. Low dark period temperature increases the critical dark period, whether the plants are young or old, by nearly 3 hours. Thus low dark period temperature seems to produce two effects; not only must the plant receive more long dark periods at a low temperature in order to be induced, but each dark period must be relatively longer than is necessary at 70° F.

EFFECT OF SATURATED ATMOSPHERE ON CRITICAL DARK PERIOD OF XANTHIUM

Of the 180 vegetative plants selected from the long photoperiod for this experiment, 130 were divided into thirteen lots of ten plants each. For the duration of the experimental period of 10 days, these were maintained during photoperiods in large glass chambers in the greenhouse, where supplementary illumination was provided. Atomizers in the chambers maintained a constant fine spray of water. In order to expose the plants to a dark period of any desired duration in a saturated atmosphere, the various lots were removed from the glass chamber at timed intervals and placed in a darkroom in which the atmosphere was kept saturated. The door to the room was provided with an adequate light and air trap. At 7:30 P.M. one lot of ten plants was removed from the glass chamber and placed in the darkroom. At 7:50 P.M. another lot was placed in the darkroom; at 8:10, another, and so on until 11:30 P.M. The whole group of 130 plants was returned to the glass chamber at 7:30 A.M. Thus the various lots received dark periods varying by 20-minute increments from 8 to 12 hours. All transfers were made as rapidly as possible.

Several determinations of the relative humidity in the darkroom were made with a psychrometer, and no wet bulb depression was observed. As far as is known, all leaves were dripping wet throughout the experimental period, except for two occasions when brief

stoppages in the atomizers in one of the glass chambers permitted the humidity to fall for a short time during two photoperiods.

The remaining fifty plants served as controls. They were divided into five lots which were left on the long photoperiod bench in the greenhouse during the photoperiods of the experimental period, and subjected to ten dark periods in an ordinary darkroom at night.

TABLE 8
RESPONSE OF XANTHIUM PLANTS IN SATURATED ATMOSPHERE
AND AT NORMAL GREENHOUSE HUMIDITIES TO
VARIOUS DARK PERIODS

ATMOSPHERIC CONDITION	LENGTH OF DARK PERIOD (HOURS)	NO. WITH IN- FLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE
Saturated or nearly so	8	0	10
	8½	0	10
	8⅔	1	9
	9	8	2
	9½	9	1
	9⅔	10	0
	10	10	0
	10½	10	0
	10⅔	10	0
	11	10	0
	11½	10	0
	11⅔	10	0
	12	10	0
Normal greenhouse humidities	8	0	10
	8½	0	10
	8⅔	10	0
	9	10	0
	9½	10	0

Dark periods were of 8 hours' duration for the first lot, and progressively longer by 20-minute increments for each of the others.

The results (table 8) indicate that very high humidities have only a minor effect on critical day length of *Xanthium*. Other experiments with low humidities (10-18 per cent) indicated similar results.

EFFECT OF LOW LIGHT INTENSITY ON CRITICAL DARK PERIOD OF XANTHIUM

Because of the somewhat irregular responses noticed at times with plants growing in the greenhouse in midwinter, it seemed possible

that low light intensity might affect critical dark period in *Xanthium*. To investigate this possibility, 180 plants were selected from those on long photoperiod. Fifty were left under full illumination as controls, and 130 were placed under a framework covered with several layers of cheesecloth, two layers of which had been tinted a light silver gray color. Light intensity was measured with a Weston light

TABLE 9
FLOWERING RESPONSE OF XANTHIUM PLANTS SUBJECTED TO
PHOTOPERIODS AT HIGH OR AT LOW LIGHT INTENSITIES TO
DARK PERIODS OF VARIOUS LENGTHS

APPROXIMATE LIGHT INTENSITY IN FOOT CANDLES DURING PHOTOPERIODS OF EXPERIMENT	DARK PERIOD DUR- ING EXPERIMENT (HOURS)	NO. WITH IN- FLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE
550	8	0	10
	8½	0	10
	8¾	2	8
	9	6	4
	9½	10	0
	9¾	10	0
	10	10	0
	10½	10	0
	10¾	10	0
	11	10	0
	11½	10	0
	11¾	10	0
	12	10	0
5500	8	0	10
	8½	9	1
	8¾	10	0
	9	10	0
	9½	10	0

meter, and at 11:00 A.M. on a clear day was found to vary at leaf surface from 500–600 foot candles on the shaded plants to 5000–6000 foot candles on the control plants. Mazda lamps were included under the cheesecloth so that the supplementary illumination was of the same duration and approximately the same intensity for experimental and control plants.

After 10 days of shading, when the plants were becoming slender and spindling, the critical dark period was determined for both groups. They were divided into lots of ten plants each, and the ex-

perimental plants given ten dark periods which varied in duration by 20-minute increments from 8 up to 12 hours. Controls were also divided into lots of ten plants each and also received ten dark periods in the same darkroom, which was maintained at approximately 70° F. The dark periods for the various lots of controls varied in duration by 20-minute increments from 8 up to $9\frac{1}{3}$ hours. After the treatment all lots, both experimental and controls, were again subdivided, half of each lot placed under the cheesecloth to develop in the shade and the other half allowed to develop under full illumination. All were dissected 2 weeks after the end of the experimental period. Although flowers developed more rapidly in those plants subjected to full illumination after photoperiodic treatment, light intensity subsequent to photoperiodic treatment did not affect the critical dark period, so the last subdivision is not indicated in table 9, where the results of this experiment are reported.

As shown in table 9, the critical dark period of the shaded plants is between $8\frac{1}{3}$ and $9\frac{1}{3}$ hours, but is difficult to determine precisely, since with dark periods of $8\frac{2}{3}$ and 9 hours some of the plants flowered and others did not. The plants grown in full sunlight had a critical dark period between 8 and $8\frac{1}{3}$ hours. Thus plants grown in shade have a somewhat longer critical dark period than plants grown in full sunlight.

Summary

1. The leaves of *Xanthium* plants are the locus of photoperiodic induction. Induced leaves, when grafted to vegetative plants, continue to supply a stimulus for flower initiation when exposed to continuous long photoperiod.

2. If during each 24-hour period, *Xanthium* plants are exposed to 15-hour dark periods at 40° F. alternating with 9-hour photoperiods at approximately 70°, then six to eight such consecutive cycles of dark period and photoperiod are required for photoperiodic induction. A treatment with four such cycles produces a residual effect which lasts more than 24 hours.

3. In these experiments, Biloxi soybeans flowered only after exposure to consecutive long dark periods, each alternating with a short photoperiod. Plants did not flower, regardless of the number of long

dark periods they received, unless at least three of the dark periods were in consecutive order.

4. Soybeans exposed to an induction period of three or more consecutive long dark periods, and which produced as a result a certain number of flower primordia, would not produce new primordia subsequently unless they were again exposed to another induction period of three or more long dark periods.

5. The length of the critical dark period for *Xanthium* is not constant. It decreases slightly in length with increasing age of the plant and varies slightly with variations in humidity. If plants are shaded during their photoperiods their critical dark periods increase slightly in length.

6. Variations in temperature greatly affect the length of the critical dark period, that of plants at 70° F. being approximately 8½ hours while that of plants at 40° is approximately 11 hours.

This investigation was carried on under the direction of and in association with Dr. KARL C. HAMNER, Department of Botany, University of Chicago.

U.S. REGIONAL SALINITY LABORATORY
RIVERSIDE, CALIFORNIA

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